Chapter 9

Microchips for Cell-Based Assays

Martin Dufva

Summary

Analysis of cells is a cornerstone in biomedical research. Traditional methods for cell culture and tissue analysis can be replaced by various microchips as discussed in this chapter. A tissue array is an example of microchip that provides higher throughput of tissue analysis. Other microchips provide completely new functionalities for the researcher. One such example that will be discussed is cell culture chips. The cell culture vessel will be much more competent for studies of cell and will enable real-time monitoring of cell behavior and gene expression at a single cell level, have possibilities for studying chemotaxis and shear stress phenomena in a controlled manner, provide least tissue-like culture conditions by providing micro 3D structures, and provide arrays of integrated detectors. Tissue arrays and the simplest forms of cell culture chips are commercialized today, and we may expect a large activity in this field in the future.

Key words: Tissue arrays, Cell culture chips

1. Introduction

Studies of cells in cultures have been done for more than 50 years. Culture of cells is traditionally done in batch culture where cells are seeded in flasks containing cell culture medium. The salinity, pH, and temperatures in the flasks are maintained in special incubators providing heat, humidity, and CO_2 control. Microtiter plates were later introduced so that throughput could be increased in biomedical research based on cell cultures. The obvious advantages with a microtiter plate are that it provides 96 (or 384) different cell cultures in a relatively small area; the volumes for each culture are relatively small leading to considerable saving of growth factors, cytokines, and other additives used for cell research, and a robotic system can be used for simplifying addition of soluble factors. However, microtiter plates are still only

providing possibilities for batch culturing and require incubators for cell maintenance. During the last decade, however, microchips for cell culture have been developed that provide a range of novel possibilities in terms of cell culture and manipulation. The strengths of these chips are that they allow experiments that are very difficult to perform in a batch culture. These novel types of experiment are possible because of the special properties of liquids when flow streams are miniaturized.

2. Effect of Miniaturization on Flow Systems

The one most notable difference between microflow systems and macroflow systems is that in microflow systems, two streams of water-based liquids do not mix to a large extent. The reason is that aqueous solutions in microsystems behave as if the aqueous solution were as thick as honey. Because of this behavior it is impossible to get turbulence in microsystems (think how difficult it would be to get turbulence in liquids as thick as honey). Two streams entering into the same channels can therefore only mix by diffusion, which is a relatively slow process. In contrast, on a macroscale, two fluids would mix fairly rapidly because of turbulence in the flow (compare how rapid mixing is obtained using a vortex). The nonturbulent flow and thereby slow mixing in microsystems can be both an advantage and a disadvantage. It is a clear disadvantage when rapid mixing is desired but because the dimensions of microsystems are usually small, mixing can be quite rapid if the flow is sufficiently slow and the molecules sufficiently small (e.g., fluorescent dyes) (**Fig. 1a**). However, mixing of proteins and DNA molecules (i.e., large molecules) by diffusion is a relatively slow process and would require very long channels (**Fig. 1b**). For example, a protein diffuses 80 µm in about 5
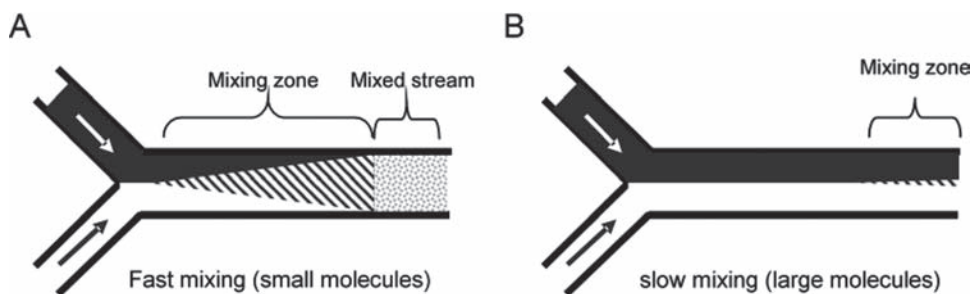

Fig. 1. Mixing in Microsystems. (**a**) Rapid diffusion of small molecules (drugs, ions gases) leads to rapid mixing of the microfluidics stream. (b) In contrast, larger molecules (DNA protein) do not efficiently mix between the two streams because of slow diffusion rate.

min. A protein solution introduced side by side with a buffer in a channel that is 160 μm × 80 μm broad and high, respectively, at a flow rate of 10 μL/min, would require a channel that is about 4 m in length before the two solutions are mixed completely. In such cases, mixing is needed to reduce channel length. Mixing can easily be obtained by patterning one side of the channel with mixer structures that resemble herring bones *(1)*. The slow mixing can, however, also be an advantage when it is desired to precisely control patterning of surfaces using flow (see later for examples).

3. Cell Culture Chips

Cell culture chips have been used for a variety of applications such as single cell observations over long periods of time *(2–4)*, study of wound healing processes *(5)*, cell–cell interactions *(6)*, differentiation *(7)*, myocyte synchronization *(8)*, chemotaxis *(9)*, electrical characterization *(10,11)*, cell stress levels *(12)*, and online monitoring of gene expression *(13)*. More advanced chambers have interconnected chambers each holding different cell lines representing the different organs and their interconnective metabolism *(14)*, and these cell culture systems are used for determining the toxicological and pharmacological profiles of chemicals and pharmaceuticals. For a comprehensive review of applications of cell culture chips, see *(15)*. Culturing cells in microfluidic devices gives a range of new possibilities to study cells that are difficult to study using standard cell culture techniques:

1. Single cells can be captured and studied individually *(2–4,16,17)*. One commercial solution is shown in **Fig. 2c** in which in the bottom of the cell culture chamber an array of small wells is micromachined. Each well can hold one cell only upon seeding and the wall makes it difficult for cells to migrate or be disturbed during staining procedures. It is therefore possible to study cell behavior in single cell level.

2. Cells can be studied in real time (see also **Fig. 3d, e**) *(18, 19)*. Real-time monitoring of cells can be performed by placing the cell culture flask under a microscope. However, only short time observation can be done because heat, humidity, and CO_2 are not usually provided. The microscope can be modified with a hood that functions as an incubator. Cells can be cultured in microtiter plates or flasks in such modified systems for days or until the medium has to be changed. There is a risk that the position of the microtiter plate or flask is disturbed
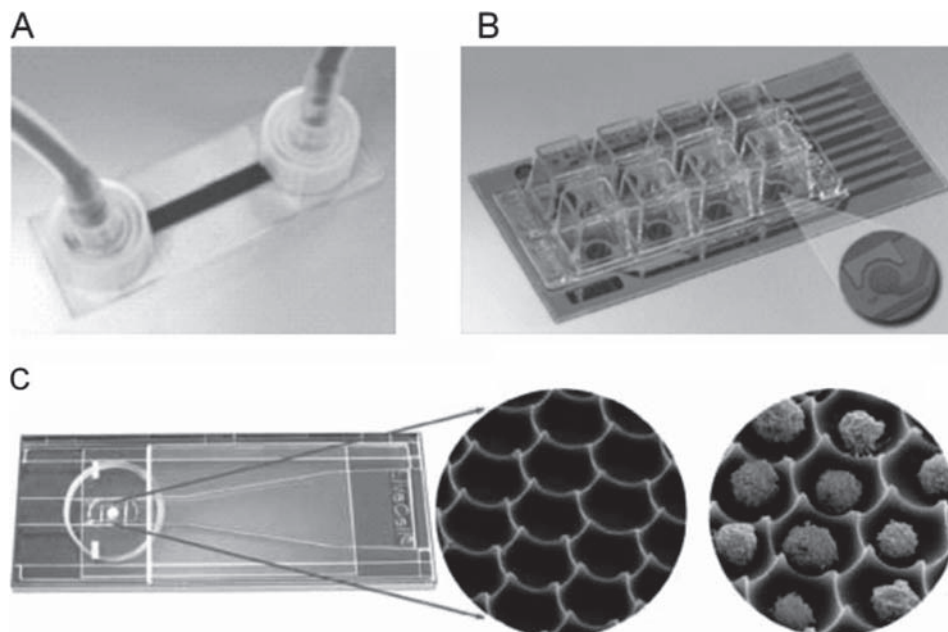
Fig. 2. Commercial cell culture chips. (**a**) flowthrough chip from Ibidi (www. Ibidi.de). (**b**) Microwell with integrated electrodes for impedance measurements (www.ibidi.de). (**c**) LiveCell Array™ from NUNC where the bottom of the microscope slides is microstructured into small wells that accept only one cell. The chip enables single cell analysis.

during medium change, and it can therefore be very difficult to study long-term effects of cells at a single cell level without flow through cell culture chips. Perfusion chips (**Fig. 2a**) can be employed instead, if longer incubation is needed to capture a response in real time. The chip displayed in **Fig. 2a** needs, however, a microscope with environmental control to sustain long-term growth. Microchips can, however, be modified with a transparent resistive film that can provide heat (*see* **Fig. 3** for an example of a chip). Because microchips as those described in **Fig. 3** are often continuously perfused with fresh medium, there is no need for controlling the CO_2 in the environment. Thus, such microchips can be used in microscopes lacking an environmental hood. Microchips for cell culturing can furthermore be constructed to have better optical properties compared with microtiter plates and flasks.

3. Fluidics controlled flow allows the performance of co-cultures. Traditionally, untreated cells (control) and treated cells (test) are contained in different flasks. Because the cultures are not 100% similar between controls and test cultures, response to a test may be difficult to find or is overexaggerated. The need for different cultures for the control and the treated cells can be circumvented using microfluidics where different parts of the same culture (bed of cells) can be treated with different solutions *(17)*.
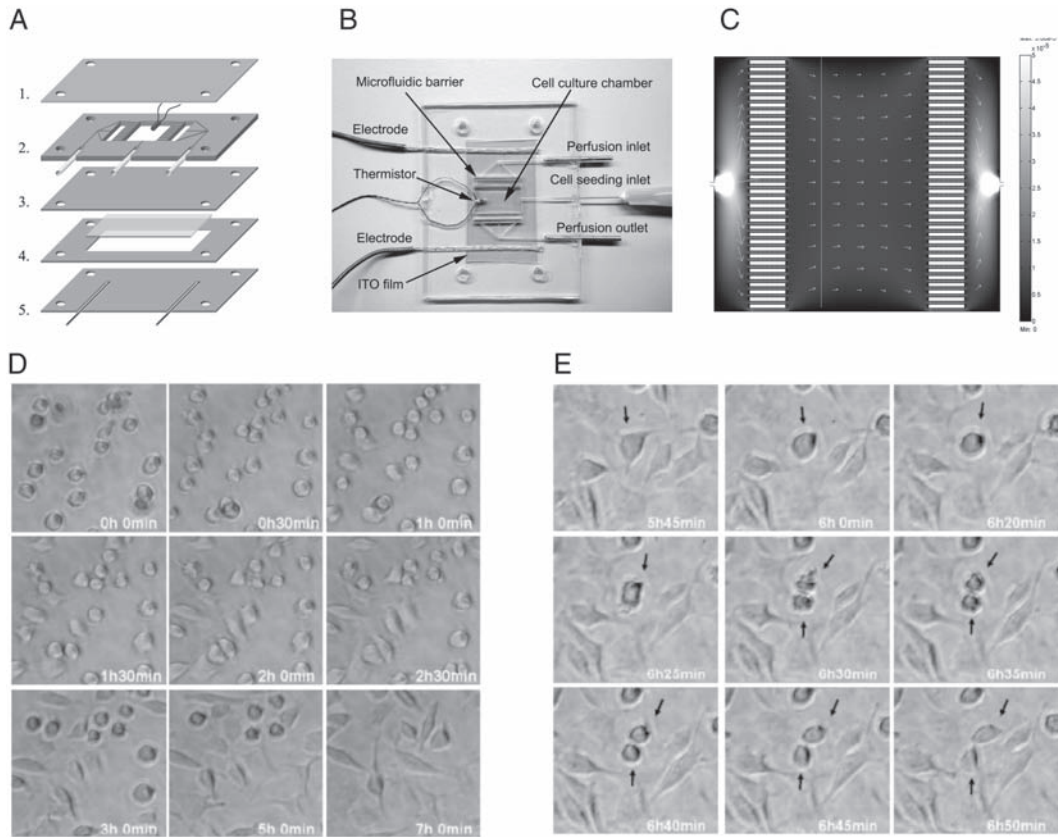
Fig. 3 Polymeric cell culture chip and filming of cells. (**a**) Parts and assembly of a polymeric cell culture chip shown in (**b**). (**c**) Simulation of nutrient distribution within the chip. Note that the distribution is quite even in the chip despite the squared form and large dimension of the chamber of 1 cm². Pictures (**a–c**) were reproduced from *(19)* (**d**). Pictures of HeLa cells taken at different time intervals after plating within the cell culture chamber. (**e**) Time-laps study of cell division.

4. Complex temporal and spatial concentration gradients can be obtained using microfluidics *(20)* allowing experiments on cells that are impossible to perform using standard methods. Simple gradients such as a gradient from a high to low concentration can also be made using macroscale technologies or simple microfluidics using for instance the chips from Ibidi (**Fig. 2a**). In the simple chips by Ibidi, the gradient is created by diffusion of substances from one inlet to the other inlet. By coupling a microfluidics network to a cell culture chip it is possible to create stable continuous chemical gradients over the bed of cell during perfusion of the cell chamber *(18)*. Such solutions allow long-time cultures of cells in a gradient of factors. More complicated gradients can furthermore be generated using microfluidics network. Li Jeon et al. for instance demonstrated that using microfluidics, a *periodic* soluble gradient of IL-8 over an area could be achieved. The

periodic gradient was used to study the migration pattern of neutrophiles in real time *(9)*. Gradients of factors can also be immobilized onto the surface of the cell culture device prior to seeding of cells. An immobilized gradient of laminin could for instance be used to study axonal outgrowth of neurons, and it was demonstrated that axonal growth was directed toward the higher laminin densities on the surface *(21)*. Another impressive example of benefits of microfluidics controlled flows was demonstrated by Lucchetta et al. who used microfluidics to perfuse a single Drosophila embryo with cold and warm medium and studied patterning of molecular markers such as Even-skipped on the embryo by time-laps microscopy, an experiment that is very difficult to perform with standard cell culture techniques. The result showed that patterning of Even-skipped could be disturbed by exposing the two different parts of the embryo to two different temperatures *(22,23)*. We can expect much more complicated experiments in the future that employ microfluidic controlled flows.

5. Microfabrication can provide microscale scaffold to promote cell growth and resemble tissue *(24–28)*. Such scaffolds are yet to be demonstrated in microtiter plates of cell culture flasks.

6. Shear stress studies can be performed because flow in shallow channels will reproducibly create shear stress.

7. Integration of other detection systems is possible using micromachined cell culture chips. A commercial example is integration of small electrodes in the bottom of a microwell (**Fig. 2b**). The electrodes can be used to study the impedance in the cell culture area. Impedance can be used to study cell growth and changes in adhesion of the cells to the surface. Impedance measurements are typically done in real time and there are no requirements that the cells are labeled. We can expect many other different sensors and actuators to be integrated into micromachined chips.

With these examples it is clear that the future cell culture chip will not only provide the basic functions for cell maintenance as microtiter wells do but also provide arrays of different detectors for studying cell responses to stimuli that are provided by combinatory microfluidics and other means.

So why is not everyone using cell culture on-chip and all the benefits that are associated with them? First, the availability of cell culture chips is limited. The commercial cell culture chips are relatively simple and if other designs and function are required, the cell chips must be fabricated in house. Moreover, many cell culture chips are made using clean room facilities (the same laboratories as for making CPUs from, e.g., Intel), which is a very expensive technology. Soft lithography of PDMS *(29)* and microfabrication of PMMA [poly(methamethyl)acrylate]

(30) are inexpensive alternatives to clean room fabricated devices but these technologies are seldom implemented in biomedical laboratories. Thus, the chips are limited to very few who have access to clean room facilities, microfabrication technologies, and knowledge of microfluidics. Cell culture chips are surprisingly easy to fabricate even for biomedical researchers. As mentioned earlier soft lithography and microfabrication of polymers are inexpensive and can be performed in a biomedical laboratory. Investments in machinery cost in the range of $30,000 for a laser to burn structures into PMMA, while spinners used for soft lithography cost much less. Pumps can be bought commercially and the simple electronics needed for on-chip heat control can be reproduced by an electronics workshop. Using relatively low technology fabrication solutions (compared to clean room facilities), it is possible to build chips for on-line monitoring of cells [**Fig. 3**; see also references *(19,31)*].

The other reason that may explain the lack of use is that the chips are not validated extensively. Usually, only a few biocompatibility parameters are tested such as adhesion, growth, and viability. It is thus not clear at present if the cell culture conditions provided on the chips are identical to the conditions that are desired, such as the condition in a cell culture flask. One dissimilarity between microchips and cell culture flasks is that microchips usually utilize perfusion to feed the cells *(16,19,24,32–34)*. In perfusion systems, the medium is always replenished, which suggests that cell growth would be better than in a standard cell culture flask. However, this is not necessarily the case, because growth factors excreted from the cells will also be washed away from the cells in perfusion systems. In some cases this can be circumvented with perfusion systems where the medium exchange is periodic. This means that the flow is stopped at time intervals to allow accumulation of growth factors. Apart from medium perfusion, cell culture chips usually are quite shallow where the height of the chamber can be as low as about 50 µm. These shallow cell culture chambers induce shear stress on the cells upon perfusion. Furthermore, oxygen or other growth factors in the medium are depleted rapidly despite the continuous flow because the volume is very small in these chips. In fact a 1-cm^2 chamber that is 50-µm high has a volume of about 5 µL. The solution is to use chambers that are higher, and it has been shown that the growth rate of the cells can be twofold higher in chambers that are 2-mm high instead of 250 µm *(35)*. At 2-mm height of the chambers, the cell growth rate is similar to the growth rate observed in cell culture flasks. A drawback with cell microchips is that the chip is fabricated in materials other than polystyrene that often is used in microtiter plates and cell culture flasks. Materials that are used are, for instance, glass, PDMS, and acrylic glass. These new surfaces have unknown effects on the cell during culture. One way to

rapidly determine effects on cells is to use gene expression profiling of cells grown on different surfaces. In a recent study, it was demonstrated that cells grown on PMMA and on modified SU8 closely resembles the gene expression of cells in cell culture flask *(36)*.

A fully validated microchip for cell culture is shown in Fig. 3. The chip is made of PMMA, which by itself does not change gene expression in HeLa cells *(36)*. It is, however, possible that just by culturing the cells in a chamber can induce changes in the cells. However, using gene expression profiling, it was shown that cells grown in that chamber showed similar expression pattern of more than 44,000 genes as cells grown in cell culture flasks *(31)*. Thus, the described cell culture chip can be viewed as a cell culture flask with the exception that the chip is perfused and can sustain cell growth for a week in a microscope that is not environmentally controlled.

4. Tissue Microarrays

Tissue microarray is a development from histological methods where, traditionally, one sample is immobilized per slide. However, the throughput of traditional method is fairly low and a way to increase throughput is to create tissue arrays consisting of up to hundreds of small samples of different tissues immobilized on a microscope slide. Such slides are tremendously powerful for analysis of expression pattern in tumors because relatively few slides can hold information from thousands of patients. In 1 day a cohort of patients could be investigated for expression of a particular marker. In comparison, using one slide per sample would require thousands of incubations, one for each patient investigated, which is labor intensive and expensive in terms of antibodies. In contrast to DNA or protein microarray, a spot in a tissue array consists of many different cells organized into a tissue. Thus, analysis of the tissue array is not simply looking on a fluorescent signal as with DNA and protein microarray. Instead, a morphological evaluation of each spot is needed, which requires that each spot is investigated manually.

5. Conclusions

It is clear that miniaturized cell analysis systems such as cell culture systems and tissue arrays will change cell and molecular biology. As opposed to cell culture chips, tissue arrays are well established

and easily adapted in existing laboratories and research. The reason is that tissue arrays are simply a higher throughput format of existing methods, while cell culture chips require heavy investment in instruments and knowledge to perform cell culture experiments in a new way.

References

1. Stroock, A.D., Dertinger, S.K., Ajdari, A., Mezic, I., Stone, H.A. and Whitesides, G.M. (2002) Chaotic mixer for microchannels. *Science*, **295**, 647–651.

2. Ince, C., Beekman, R.E. and Verschragen, G. (1990) A micro-perfusion chamber for single-cell fluorescence measurements. *J Immunol Methods*, **128**, 227–234.

3. Jager, E.W.H., Immerstrand, C., Peterson, K.H., Magnusson, K.-E., Lundström, I. and Inganäs, O. (2002) The cell clinic: closable microvials for single cell studies. *Biomedical Microdevices*, **4**, 177–187.

4. Prokop, A., Prokop, Z., Schaffer, D., Kozlov, E., Wikswo, J., Cliffel, D. and Baudenbacher, F. (2004) NanoLiterBioReactor: long-term mammalian cell culture at nanofabricated scale. *Biomed Microdevices*, **6**, 325–339.

5. Ho, C.L., Mou, T.Y., Chiang, P.S., Weng, C.L. and Chow, N.H. (2005) Mini chamber system for long-term maintenance and observation of cultured cells. *Biotechniques*, **38**, 267–273.

6. Moriguchi, H., Wakamoto, Y., Sugio, Y., Takahashi, K., Inoue, I. and Yasuda, K. (2002) An agar-microchamber cell-cultivation system: flexible change of microchamber shapes during cultivation by photo-thermal etching. *Lab Chip*, **2**, 125–132.

7. Tourovskaia, A., Figueroa-Masot, X. and Folch, A. (2005) Differentiation-on-a-chip: a microfluidic platform for long-term cell culture studies. *Lab Chip*, **5**, 14–19.

8. Kojima, K., Kaneko, T. and Yasuda, K. (2004) A novel method of cultivating cardiac myocytes in agarose microchamber chips for studying cell synchronization. *J Nanobiotechnol*, **2**, 9.

9. Li Jeon, N., Baskaran, H., Dertinger, S.K., Whitesides, G.M., Van de Water, L. and Toner, M. (2002) Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat Biotechnol*, **20**, 826–830.

10. Blau, A.W. and Ziegler, C.M. (2001) Prototype of a novel autonomous perfusion chamber for long-term culturing and in situ investigation of various cell types. *J Biochem Biophys Methods*, **50**, 15–27.

11. Hediger, S., Fontannaz, J., Sayah, A., Hunziker, W. and Gijs, M.A.M. (2000) Biosystem for the culture and characterisation of epithelial cell tissues. *Sens Actuators B Chem*, **63**, 63–73.

12. Davidsson, R., Boketoft, A., Bristulf, J., Kotarsky, K., Olde, B., Owman, C., Bengtsson, M., Laurell, T. and Emneus, J. (2004) Developments toward a microfluidic system for long-term monitoring of dynamic cellular events in immobilized human cells. *Anal Chem*, **76**, 4715–4720.

13. Thompson, D., King, K., Wieder, K., Toner, M., Yarmush, M. and Jayaraman, A. (2004) Dynamic gene expression profiling using a microfabricated living cell array. *Anal Biochem*, **76**, 4098–4103.

14. Sin, A., Chin, K.C., Jamil, M.F., Kostov, Y., Rao, G. and Shuler, M.L. (2004) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. *Biotechnol Prog*, **20**, 338–345.

15. El-Ali, J., Sorger, P.K. and Jensen, K.F. (2006) Cells on chips. *Nature*, 442, 403–411.

16. Werdich, A.A., Lima, E.A., Ivanov, B., Ges, I., Anderson, M.E., Wikswo, J.P. and Baudenbacher, F.J. (2004) A microfluidic device to confine a single cardiac myocyte in a sub-nanoliter volume on planar microelectrodes for extracellular potential recordings. *Lab Chip*, **4**, 357–362.

17. Kaji, H., Nishizawa, M. and Matsue, T. (2003) Localized chemical stimulation to micropatterned cells using multiple laminar fluid flows. *Lab Chip*, **3**, 208–211.

18. Chung, B.G., Flanagan, L.A., Rhee, S.W., Schwartz, P.H., Lee, A.P., Monuki, E.S. and Jeon, N.L. (2005) Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip*, **5**, 401–406.

19. Petronis, S., Stangegaard, M., Christensen, C. and Dufva, M. (2006) Transparent polymeric cell culture chip with integrated temperature

control and uniform media perfusion. *Biotechniques*, **40**, 368–376.

20. Chung, B.G., Lin, F. and Jeon, N.L. (2006) A microfluidic multi-injector for gradient generation. *Lab Chip*, **6**, 764–768.

21. Dertinger, S.K., Jiang, X., Li, Z., Murthy, V.N. and Whitesides, G.M. (2002) Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc Natl Acad Sci USA*, **99**, 12542–12547.

22. Lucchetta, E.M., Lee, J.H., Fu, L.A., Patel, N.H. and Ismagilov, R.F. (2005) Dynamics of Drosophila embryonic patterning network perturbed in space and time using microfluidics. *Nature*, **434**, 1134–1138.

23. Lucchetta, E.M., Munson, M.S. and Ismagilov, R.F. (2006) Characterization of the local temperature in space and time around a developing Drosophila embryo in a microfluidic device. *Lab Chip*, **6**, 185–190.

24. Powers, M.J., Domansky, K., Kaazempur-Mofrad, M.R., Kalezi, A., Capitano, A., Upadhyaya, A., Kurzawski, P., Wack, K.E., Stolz, D.B., Kamm, R.et al. (2002) A microfabricated array bioreactor for perfused 3D liver culture. *Biotechnol Bioeng*, **78**, 257–269.

25. Bianchi, F., Rosi, M., Vozzi, G., Emanueli, C., Madeddu, P. and Ahluwalia, A. (2006) Microfabrication of fractal polymeric structures for capillary morphogenesis: applications in therapeutic angiogenesis and in the engineering of vascularized tissue. *J Biomed Mater Res B Appl Biomater*

26. Mapili, G., Lu, Y., Chen, S. and Roy, K. (2005) Laser-layered microfabrication of spatially patterned functionalized tissue-engineering scaffolds. *J Biomed Mater Res B Appl Biomater*, **75**, 414–424.

27. Norman, J.J. and Desai, T.A. (2005) Control of cellular organization in three dimensions using a microfabricated polydimethylsiloxane–collagen composite tissue scaffold. *Tissue Eng*, **11**, 378–386.

28. Petronis, S., Eckert, K.L., Gold, J. and Wintermantel, E. (2001) Microstructuring ceramic scaffolds for hepatocyte cell culture. *J Mater Sci Mater Med*, **12**, 523–528.

29. McDonald, J.C., Duffy, D.C., Anderson, J.R., Chiu, D.T., Wu, H., Schueller, O.J. and Whitesides, G.M. (2000) Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis*, **21**, 27–40.

30. Klank, H., Kutter, J.P. and Geschke, O. (2002) CO(2)-laser micromachining and back-end processing for rapid production of PMMA-based microfluidic systems. *Lab Chip*, **2**, 242–246.

31. Stangegaard, M., Petronis, S., Jorgensen, A.M., Christensen, C.B. and Dufva, M. (2006) A biocompatible micro cell culture chamber (microCCC) for the culturing and on-line monitoring of eukaryote cells. *Lab Chip*, **6**, 1045–1051.

32. Hung, P.J., Lee, P.J., Sabounchi, P., Aghdam, N., Lin, R. and Lee, L.P. (2005) A novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array. *Lab Chip*, **5**, 44–48.

33. Hung, P.J., Lee, P.J., Sabounchi, P., Lin, R. and Lee, L.P. (2005) Continuous perfusion microfluidic cell culture array for high-throughput cell-based assays. *Biotechnol Bioeng*, **89**, 1–8.

34. Fidkowski, C., Kaazempur-Mofrad, M.R., Borenstein, J., Vacanti, J.P., Langer, R. and Wang, Y. (2005) Endothelialized microvasculature based on a biodegradable elastomer. *Tissue Eng*, **11**, 302–309.

35. Yu, H., Meyvantsson, I., Shkel, I.A. and Beebe, D.J. (2005) Diffusion dependent cell behavior in microenvironments. *Lab Chip*, **5**, 1089–1095.

36. Stangegaard, M., Wang, Z., Kutter, J.P., Dufva, M. and Wolff, A. (2006) Whole genome expression profiling using DNA microarray for determining biocompatibility of polymeric surfaces. *Mol Biosyst*, **2**, 421–428.